Monitor for Sterilization Procedures

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Abstract – **Rapid and accurate determination of the efficacy of sterilization procedures is demonstrated using a hand-held instrument based on the intrinsic fluorescence of germinating spores. This technology requires no added reagents or sample contact.**

I. Introduction

In a recent report on infectious disease [1], the World Health Organization cites hospital-acquired 'super-infections', multi-drug resistant infections, as a major threat to world health. For example, in the US alone, treatment of hospital-acquired multi-resistant infections costs over $10 billon per year with the result of over 14,000 deaths. Furthermore, such infections can migrate to the community at large. Unless, we slow the spread of resistant bacteria, even commonplace procedures like dental surgery will be high risk. The ramifications of this are unimaginable. Inadequately cleaned equipment is cited as a major determinant in the spread of infectious disease.

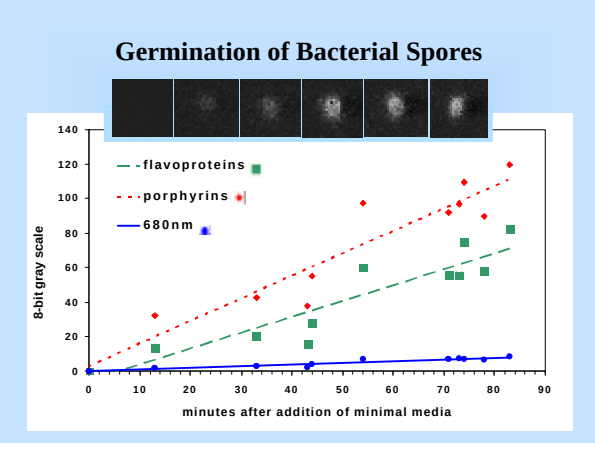
Current diagnostic technologies [2] require outgrowth of the microbes in the detection protocols, a step which requires hours to days. Unfortunately, many facilities do not have this time before emergencies force the use of sterilized equipment. They may also not have the capability to keep the equipment sterile while they await the results of the efficacy of their sterilization procedures. In view of the risk to human life, this uncertainty must be removed.

Intrinsic fluorescence of bacteria offers a detection method that has high sensitivity, short collection time requirements, no sample contact, and the capability of scanning surfaces. Furthermore, the fluorescence intensity is proportional to the excitation intensity, so high-power illumination can be used to observe weak signals. Redox fluorimetry based on intrinsic fluorescence markers has proved to be a vital tool in the investigation of cellular metabolism and tissue oxygenation [3].

We have characterized the appearance of intrinsic fluorescence on spore germination and used these results to develop methods and prototype devices for the detection of bacterial life on surfaces, such as glass and stainless steel [4].

II. Characterization of Intrinsic Fluorescence on Spore Germination

All living cells must perform respiration to produce energy from food. There are several steps in this biochemical process which produce intermediate products [metabolites] that fluoresce. Three such metabolites are the reduced pyridine nucleotides, flavoproteins, and porphyrins. We have characterized these metabolites in the process of spore germination using standard light and confocal microscopy. The confocal microscope provides clearer, better-defined images of bacterial cells and spores as well as quantitative fluorescence measurements. The results summarized in the figure below were obtained with *Bacillus subtilis* spores after the addition of a minimal media that had low fluorescence. It is important that the production of metabolites [flavoproteins and porphyrins, 680 nm measurement is the control] is observed within minutes of addition of the minimal media, even though the appearance is that of a spore. This production of metabolites increases as the spore becomes a vegetative cell and finally a mature rod-shaped cell. Thus, by the observation of



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Abstract

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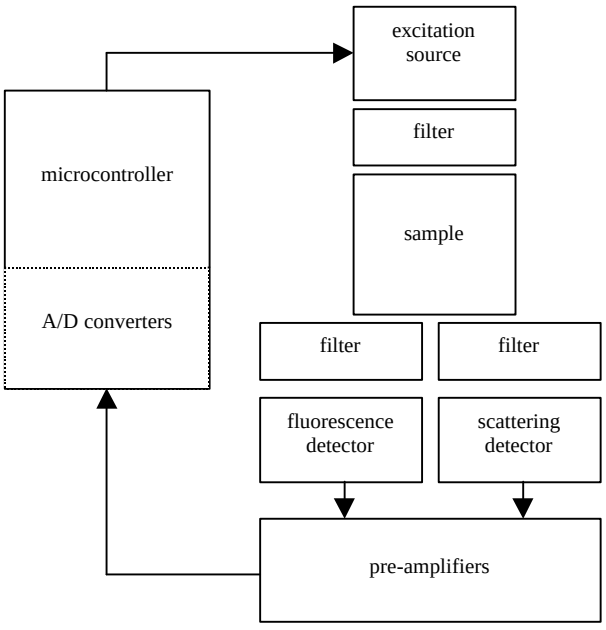
metabolite fluorescence, we can detect the presence of viable spores and cells in a few minutes.

III. Hand-held prototype instrument

A hand-held prototype instrument was constructed based on these results as shown in the block diagram below. Measurement of flavoprotein and/or porphyrin fluorescence is made and normalized by the scattering of the incident light. This approximately cancels the optical differences, which occur in different samples under varying conditions [e.g., scattering characteristics of the matrix, distance from the source of the fluorescence signals, penetration depth]. The environmental background is measured in the off-cycle of the pulsed LED light source, which allows contributions from other light sources to be subtracted from each measurement pulse. The fluorescence and scattered light are separated by filters and detected by photomultiplier tubes [e.g., Hammatsu H6780 series]. These signals are integrated over the pulse width, amplified using specially-designed, high-gain, low-noise analog circuits [see A. Duncan and L. Powers, these proceedings], and converted to a digital signal. The microcontroller takes the appropriate signal ratios, which are displayed on an LCD, and controls the pulse width and repetition rate of the LED light source.



IV. Calibration and testing

This method and prototype instrument were tested using ampules of *Bacillus stearothermophilus* spores commercially manufactured by Barnstead/Thermolyne for testing sterilization procedures. Instead of out-growth after sterilization, the spores were placed in our non-fluorescent minimal media and measured with the prototype instrument. Germination could be detected after minutes in spores that had undergone incomplete sterilization procedures. Microbial contamination on medical instruments that had been exposed to spores or viable cells could also be detected.

V. Conclusions

In summary, detection of germination of spore samples used as controls to determine the efficacy of sterilization procedures can be made in minutes with this technology in minutes after the sterilization procedure is completed. In addition, bacterial contamination on medical instruments and surfaces can also be assessed.

References

[1] Leidl, P. (2000), Overcoming Antimicrobial Resistance, World Health Organization Report on Infectious Diseases 2000, World Health Organization.

[2] Hobson, N., Tothill, I., and Turner, A. (1996), Microbial Detection, Biosens. & Bioelectron, 11, 455.

[3] Barlow, C. and Chance, B. (1976), Ischemic Areas in Perfused Rat Hearts: Measurement by NADH Fluorescence Photography, Science 193, 909.

[4] Estes, C., Duncan, A., Wade, B., and Powers, L., Real-Time Multiwavelength Microbe Detection Instrument, Abstract for Poster Presentation at the EMBS/BMES Joint Conference, Atlanta, GA, Oct. 13-16, 1999.